

CLAIMS

1. An isolated polypeptide having the ability to bind to fibrin comprising the amino acid sequence: Cys-X₂-X₃-X₄-X₅-X₆-X₇-X₈-Cys (SEQ ID NO: 2), wherein X₂ is Pro, Arg, Asn, Asp, Gln, Gly, Phe, Ser, Thr or Tyr; X₃ is Ala, Asn, Asp, Gln, Glu, Gly, Ile, Leu, Met, Phe, Pro, Ser, Thr, Trp, Tyr, or Val; X₄ is Glu, Gly, Lys, Ser, or Tyr; X₅ is Pro, Asp, Glu, Asn, Gln, Glu, Gly, Leu, Lys, Ser, Thr, or Tyr; X₆ is Arg, Gly, or Trp; X₇ is Leu, Ile, Lys, Met, Asn, Gln, Pro, Ser, Thr, or Val; and X₈ is Ile, Leu, Phe, Trp, or Tyr.
2. The polypeptide according to Claim 1, comprising the amino acid sequence: Cys-X₂-X₃-X₄-X₅-Trp-X₇-X₈-Cys (SEQ ID NO: 42), wherein X₂ is Pro, Asn, Gln, Ser, or Thr; X₃ is Ala, Asn, Asp, Gln, Glu, Gly, Ile, Leu, Met, Phe, Pro, Ser, Thr, Trp, Tyr, or Val; X₄ is Glu or Ser; X₅ is Pro, Asp, Glu, Asn, Gln, Ser, Thr, or Tyr; X₇ is Leu, Ile, Met, Asn, Gln, Ser, Thr, or Val; and X₈ is Phe, Trp, or Tyr.
3. The polypeptide according to Claim 1, wherein the following amino acid positions are independently selected as follows: the amino acid residue X₂ is Pro, the amino acid residue X₃ is Asp, Glu, Gly, Met, or Trp, the amino acid residue X₄ is Glu, the amino acid residue X₅ is Asn, Asp, Glu, Pro, or Ser, the amino acid residue X₆ is Trp, the amino acid residue X₇ is Leu or Thr, the amino acid residue X₈ is Phe, or combinations of such selections.

4. The polypeptide according to Claim 1, wherein the polypeptide comprises an amino acid sequence selected from the group consisting of:

Cys-Ser-Asp-Glu-Asn-Trp-Leu-Trp-Cys (SEQ ID NO: 21),
Cys-Pro-Met-Ser-Glu-Trp-Leu-Tyr-Cys (SEQ ID NO: 22),
Cys-Pro-Trp-Glu-Ser-Trp-Thr-Phe-Cys (SEQ ID NO: 23),
Cys-Gln-Glu-Glu-Pro-Trp-Leu-Phe-Cys (SEQ ID NO: 24),
Cys-Pro-Gly-Glu-Asp-Trp-Leu-Phe-Cys (SEQ ID NO: 25),
Cys-Tyr-Gly-Glu-Ser-Gly-Ile-Phe-Cys (SEQ ID NO: 43);
Cys-Thr-Gly-Glu-Pro-Gly-Pro-Ile-Cys (SEQ ID NO: 44);
Cys-Gln-Leu-Gly-Tyr-Arg-Thr-Tyr-Cys (SEQ ID NO: 45);
Cys-Asp-Gly-Glu-Pro-Trp-Leu-Phe-Cys (SEQ ID NO: 46);
Cys-Gly-Trp-Gly-Ser-Trp-Lys-Phe-Cys (SEQ ID NO: 47);
Cys-Gly-Trp-Gly-Ser-Gly-Lys-Leu-Cys (SEQ ID NO: 48);
Cys-Pro-Gly-Glu-Pro-Trp-Thr-Phe-Cys (SEQ ID NO: 49);
Cys-Pro-Gly-Glu-Pro-Trp-Thr-Phe-Cys (SEQ ID NO: 50);
Cys-Pro-Gly-Tyr-Leu-Arg-Ser-Leu-Cys (SEQ ID NO: 51);
Cys-Pro-Gly-Glu-Pro-Trp-Ser-Phe-Cys (SEQ ID NO: 52);
Cys-Arg-Gly-Glu-Ser-Trp-Pro-Tyr-Cys (SEQ ID NO: 53);
Cys-Pro-Gly-Tyr-Lys-Arg-Gln-Phe-Cys (SEQ ID NO: 54);
Cys-Gly-Gln-Glu-Ser-Arg-Thr-Phe-Cys (SEQ ID NO: 55); and
Cys-Phe-Gln-Lys-Gly-Gly-Thr-Leu-Cys (SEQ ID NO: 56).

5. A fibrin binding polypeptide comprising the amino acid sequence: Cys-Asp-Tyr-Tyr-

Gly-Thr-Cys (SEQ ID NO: 26).

6. The polypeptide according to Claim 1, comprising the amino acid sequence:

$X_1-X_2-X_3-X_4-X_5-X_6-X_7-X_8-X_9-X_{10}-X_{11}-X_{12}-X_{13}-X_{14}-X_{15}$ (SEQ ID NO: 1),

wherein

X_1 is Cys, Pro, or Trp;

X_2 is Ala, Arg, Asn, Asp, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr or Val, or if X_4 and X_{12} are not Cys, then X_2 may be Cys;

X_3 is Ala, Asn, Gln, Gly, Ile, Leu, Met, Phe, Pro, or Thr;
 X_4 is Cys or another amino acid capable of forming a covalent cross-link to X_{12} ;
 X_5 is Pro, Arg, Asn, Asp, Gln, Gly, Phe, Ser, Thr or Tyr;
 X_6 is Ala, Asn, Asp, Gln, Glu, Gly, Ile, Leu, Met, Phe, Pro, Ser, Thr, Trp, Tyr, or Val;
 X_7 is Glu, Gly, Lys, Ser, or Tyr;
 X_8 is Pro, Asp, Glu, Asn, Gln, Glu, Gly, Leu, Lys, Ser, Thr, or Tyr;
 X_9 is Arg, Gly, or Trp;
 X_{10} is Leu, Ile, Lys, Met, Asn, Gln, Pro, Ser, Thr, or Val;
 X_{11} is Ile, Leu, Phe, Trp, or Tyr;
 X_{12} is Cys or another amino acid capable of forming a covalent cross-link to X_4 ;
 X_{13} is Cys, Gly, Leu, Phe, Pro, Trp, or Tyr;
 X_{14} is Pro, Ala, Gly, Asn, Gln, Lys, Ser, Thr, Tyr, Asp, Glu, or His; and
 X_{15} is Ala, Arg, Asp, Ile, Leu, Met, Phe, Pro, Trp, Val, Asn, Gln, Gly, Ser, Thr, Tyr, or His.

7. The polypeptide according to Claim 6, comprising the amino acid sequence:

Trp- X_2 - X_3 - X_4 - X_5 - X_6 - X_7 - X_8 -Trp- X_{10} - X_{11} - X_{12} - X_{13} - X_{14} - X_{15} (SEQ ID NO:41),
wherein

X_2 is Ala, Arg, Asn, Asp, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr or Val, or if X_4 and X_{12} are not Cys, then X_2 may be Cys;

X_3 is Ala, Asn, Gln, Gly, Ile, Leu, Met, Phe, or Pro;

X_4 is Cys or another amino acid capable of forming a covalent cross-link to X_{12} ;

X_5 is Pro, Asn, Gln, Ser, or Thr;

X_6 is Ala, Asn, Asp, Gln, Glu, Gly, Ile, Leu, Met, Phe, Pro, Ser, Thr, Trp, Tyr, or Val;

X_7 is Glu or Ser;

X_8 is Pro, Asp, Glu, Asn, Gln, Ser, Thr, or Tyr;

X_{10} is Leu, Ile, Met, Asn, Gln, Ser, Thr, or Val;

X_{11} is Phe, Trp, or Tyr;

X_{12} is Cys or another amino acid capable of forming a covalent cross-link to X_4 ;

X₁₃ is Phe, Trp, or Tyr;
X₁₄ is Pro, Ala, Gly, Asn, Gln, Ser, Thr, Tyr, Asp, Glu, or His; and
X₁₅ is Ala, Ile, Leu, Met, Phe, Pro, Trp, Val, Asn, Gln, Gly, Ser, Thr, Tyr, or His.

8. The polypeptide according to Claim 7, wherein the following amino acid positions are independently selected as follows: the amino acid residue X₂ is Ala, Gln, Glu, Lys, or Met; the amino acid residue X₃ is Ala, Leu, Met, or Pro; the amino acid residue X₄ is Cys; the amino acid residue X₅ is Pro; the amino acid residue X₆ is Asp, Glu, Gly, Met, or Trp; the amino acid residue X₇ is Glu; the amino acid residue X₈ is Asn, Asp, Glu, Pro, or Ser; the amino acid residue X₁₀ is Leu or Thr; the amino acid residue X₁₁ is Phe; the amino acid residue X₁₂ is Cys; the amino acid residue X₁₃ is Trp; the amino acid residue X₁₄ is Asp, Gly, His, Phe, or Ser; the amino acid residue X₁₅ is Ala, Gly, His, Pro, or Ser, or combinations of such selections.
9. The polypeptide according to Claim 8, wherein the following amino acid positions are independently selected as follows: the amino acid residue X₅ is Pro, the amino acid residue X₇ is Glu, the amino acid residue X₁₀ is Leu, the amino acid residue X₁₁ is Phe, the amino acid residue X₁₃ is Trp, or combinations of such selections.
10. The polypeptide according to Claim 6, wherein the polypeptide comprises an amino acid sequence selected from the group consisting of:
Trp-Glu-Leu-Cys-Ser-Asp-Glu-Asn-Trp-Leu-Trp-Cys-Trp-Pro-His (SEQ ID NO: 3),
Trp-Met-Met-Cys-Pro-Met-Ser-Glu-Trp-Leu-Tyr-Cys-Trp-Ser-Ala (SEQ ID NO: 4),
Trp-Gln-Pro-Cys-Pro-Trp-Glu-Ser-Trp-Thr-Phe-Cys-Trp-Asp-Pro (SEQ ID NO: 5),
Trp-Ala-Pro-Cys-Gln-Glu-Glu-Pro-Trp-Leu-Phe-Cys-Phe-His-Gly (SEQ ID NO: 6),
Trp-Lys-Ala-Cys-Pro-Gly-Glu-Asp-Trp-Leu-Phe-Cys-Trp-Gly-Ser (SEQ ID NO: 7),
Pro-Arg-Pro-Cys-Tyr-Gly-Glu-Ser-Gly-Ile-Phe-Cys-Trp-Lys-Val (SEQ ID NO: 27);
Pro-Arg-Pro-Cys-Thr-Gly-Glu-Pro-Gly-Pro-Ile-Cys-Gly-Pro-Arg (SEQ ID NO: 28);
Trp-Gln-Ala-Cys-Gln-Leu-Gly-Tyr-Arg-Thr-Tyr-Cys-Trp-Asp-Gly (SEQ ID NO: 29);
Trp-Lys-Phe-Cys-Asp-Gly-Glu-Pro-Trp-Leu-Phe-Cys-Trp-Asp-Gly (SEQ ID NO: 30);

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Trp-Asn-Gly-Cys-Gly-Trp-Gly-Ser-Trp-Lys-Phe-Cys-Gly-Glu-Gly (SEQ ID NO:31);
Trp-Leu-Asn-Cys-Gly-Trp-Gly-Ser-Gly-Lys-Leu-Cys-Leu-Gly-Val (SEQ ID NO:32);
Cys-Tyr-Phe-Cys-Pro-Gly-Glu-Pro-Trp-Thr-Phe-Cys-Cys-Asp-Asp (SEQ ID NO:33);
Trp-His-Phe-Cys-Pro-Gly-Glu-Pro-Trp-Thr-Phe-Cys-Trp-Ala-Gly (SEQ ID NO:34);
Trp-Gln-Thr-Cys-Pro-Gly-Tyr-Leu-Arg-Ser-Leu-Cys-Trp-Asp-Gly (SEQ ID NO:35);
Trp-Tyr-Phe-Cys-Pro-Gly-Glu-Pro-Trp-Ser-Phe-Cys-Pro-Asp-Gly (SEQ ID NO:36);
Pro-Arg-Pro-Cys-Arg-Gly-Glu-Ser-Trp-Pro-Tyr-Cys-Trp-Gly-Gly (SEQ ID NO:37);
Trp-Gln-Ala-Cys-Pro-Gly-Tyr-Lys-Arg-Gln-Phe-Cys-Trp-Asp-Arg (SEQ ID NO:38);
Pro-Arg-Pro-Cys-Gly-Gln-Glu-Ser-Arg-Thr-Phe-Cys-Leu-Glu-Gly (SEQ ID NO:39);
and
Pro-Arg-Pro-Cys-Phe-Gln-Lys-Gly-Gly-Thr-Leu-Cys-Trp-Pro-Gly (SEQ ID NO:40).

11. The polypeptide according to Claim 5, having the amino acid sequence: Arg-Ala-Pro-Cys-Asp-Tyr-Tyr-Gly-Thr-Cys-Val-Glu-Leu (SEQ ID NO: 8).
12. A method of detecting fibrin in a mammalian subject comprising the steps of:
 - (a) detectably labeling a polypeptide according to any one of Claims 1-11;
 - (b) administering to said subject the labeled polypeptide and, thereafter,
 - (c) detecting the labeled polypeptide in the subject.
13. The method according to Claim 12, wherein said label is fluorescent, echogenic, radioactive or paramagnetic.
14. The method according to Claim 12, wherein said label is ^{111}In or $^{99\text{m}}\text{Tc}$.
15. The method of according to Claim 12, wherein said detecting step is indicative of deep-vein thrombosis, pulmonary embolism, cardiogenic thrombosis, atherosclerosis or stroke.

16. A method of treating a disease involving thrombus formation, comprising the step: administering to a mammalian subject in need of treatment for such a disease a composition comprising a polypeptide according to any one of Claims 1-11 conjugated with a pharmaceutical effective for treating said disease involving thrombus formation.

17. The method according to Claim 16, wherein said disease is deep-vein thrombosis, pulmonary embolism, cardiogenic thrombosis, atherosclerosis, myocardial infarct, reperfusion ischemia, or stroke.

18. The method according to Claim 16, wherein said pharmaceutical is a thrombolytic agent selected from tPA, streptokinase, and urokinase.

19. A recombinant host cell or bacteriophage expressing on its surface an exogenous fibrin binding polypeptide according to any one of Claims 1-11.

20. A magnetic resonance imaging contrast agent comprising at least one paramagnetic metal atom linked to at least one polypeptide according to any one of Claims 1-11.

21. The magnetic resonance imaging contrast agent according to Claim 20, wherein said magnetic resonance imaging contrast agent further comprises at least one chelator selected from the group consisting of DTPA, DOTA, EDTA, TETA, EHPG, HBED, NOTA, DOTMA, TETMA, PDTA, TTHA, LICAM, and MECAM.

22. The magnetic resonance imaging contrast agent according to Claim 21, wherein said chelator comprises diethylenetriamine or tetraazacyclododecane or a carboxymethyl-substituted derivative thereof.

23. The magnetic resonance imaging contrast agent according to Claim 21, wherein said paramagnetic metal atom is selected from the group consisting of: Mn^{2+} , Cu^{2+} , Fe^{2+} , Co^{2+} , Ni^{2+} , Gd^{3+} , Eu^{3+} , Dy^{3+} , Pr^{3+} , Cr^{3+} , Co^{3+} , Fe^{3+} , Ti^{3+} , Tb^{3+} , Nd^{3+} , Sm^{3+} , Ho^{3+} ,

Er^{3+} , Pa^{4+} , and Eu^{2+} .

24. The magnetic resonance imaging contrast agent according to Claim 23, wherein said paramagnetic metal atom is Gd^{3+} .
25. A method for identifying fibrin binding compounds comprising the steps of utilizing a fibrin binding polypeptide according to any one of Claims 1–11 to form a complex with a fibrin target, contacting said complex with one or more potential fibrin binding compounds, and determining whether said one or more potential fibrin binding compounds competes with said fibrin binding polypeptide to form a complex with said fibrin target.
26. A method for identifying fibrin binding compounds comprising the steps of contacting a solution containing a potential fibrin binding compound with fibrin target to form a complex between said compound and the fibrin target, contacting said complex with a fibrin binding polypeptide according to any one of Claims 1–11, and determining whether said fibrin binding polypeptide competes with said potential fibrin binding compound to form a complex with said fibrin target.
27. A diagnostic imaging agent comprising a polypeptide according to any one of Claims 1–11 linked to a detectable label.
28. The imaging agent according to Claim 27, wherein said polypeptide is radiolabeled.
29. The imaging agent according to Claim 27, wherein said polypeptide is labeled with $^{99\text{m}}\text{Tc}$.
30. The imaging agent according to Claim 27, wherein said polypeptide is fluoresceinated.
31. The imaging agent according to Claim 27, wherein said polypeptide is linked to an echogenic label suitable for ultrasound imaging.

32. A method of medical imaging comprising the steps of administering to a mammalian subject a pharmaceutical preparation of a contrast agent comprising at least one polypeptide according to any one of Claims 1–11 and imaging said contrast agent by a step selected from the group consisting of magnetic resonance imaging, ultrasound imaging, optical imaging, sonoluminescence imaging, photoacoustic imaging, and nuclear imaging.
33. The method of medical imaging according to Claim 32, wherein said administering step is selected from among the group consisting of: inhaling, transdermal absorbing, intramuscular injecting, subcutaneous injecting, intravenous injecting, and intra-arterial injecting.
34. The method of medical imaging according to Claim 32, wherein said pharmaceutical preparation is packaged in a container selected from among the group consisting of: kit, syringe, vial, bottle, flexible container, packet, or inhaler.
35. A method of purifying fibrin or fibrin-like polypeptide from a solution containing it comprising contacting the solution with at least one polypeptide according to any one of Claims 1–11, and then separating said polypeptide from said solution.